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Neisseria gonorrhoeae: subdivision of auxogroups by genetic transformation

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SUMMARY Genetic transformation was used in an attempt to subdivide the most prevalent auxotypes of *Neisseria gonorrhoeae* in local isolates. The large proline requiring (Pro^-) group could be divided into two genetic types, as could the less common arginine requiring (Arg^-) group. The large arginine, hypoxanthine, and uracil requiring ($Arg^- Hyp^- Ura^-$) group could not be subdivided by this method. The genetic relation between these and other auxotypes was investigated.

Introduction

Since the publication of Catlin's chemically defined medium in 1973,[1] auxotyping has been applied widely in epidemiological studies of *Neisseria gonorrhoeae*. Requirements for proline (Pro^-), arginine (Arg^-), hypoxanthine (Hyp^-), and uracil (Ura^-) have been found empirically to be the most useful markers. By including an additional arginine free medium that has been supplemented with ornithine, the arginine requirement can be subdivided into an arginine requirement satisfied by ornithine (Arg^-) and an arginine requirement not satisfied by ornithine (Arg°).

A few auxotypes often account for a large proportion of clinical isolates.[2-4] In Avon the prototrophic group (NR, non-requiring), Pro^- strains, and $Arg^- Hyp^- Ura^-$ strains accounted for over 70% of clinical isolates between 1982 and 1984.[5]

Using genetic transformation other workers have shown that some phenotypically identical auxotypes can be caused by different genetic defects.[6] Here this technique has been applied to local clinical isolates in an attempt to subdivide the most common auxotrophic groups and to investigate the genetic relation between these and other auxogroups.

Materials and methods

STRAINS OF *NEISSERIA GONORRHOEAE*

The isolates used in this study are described in table I.

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Recipient strains

Fresh isolates were selectively subcultured on Kellogg's typing medium for T 1 or T 2 colony types.[7] When cultures producing more than 95% of the desired colony type were achieved the strain was suspended in horse serum and stored at -80°C. Recipient strains were selected from this collection on the basis of auxotype, the ability to be transformed from prototrophic strains by deoxyribonucleic acid (DNA), and the stability of characteristics after subculture.

TABLE I *Clinical isolates used in this study*

Auxotype	*No of strains*	*Designation*	*Used as:*
NR	4	NR 1-4	Donor
Pro^-	2	P 1-2	Recipient & donor
Pro^- (1982)	8	P 3-10	Donor
Pro^- (1983)	18	P 11-28	Donor
Pro^- (1985)	36	P 29-64	Donor
$Pro^- Arg^-$	2	PA 1-2	Donor
$Pro^- Arg^\circ Ura^-$	3	PA°U 1-3	Recipient & donor
$Pro(oc)^-$	3	Poc 1-3	Donor
Arg^-	1	A 1	Recipient
Arg^-	6	A 2-7	Donor
$Arg^- Hyp^- Ura^-$	2	AHU 1-2	Recipient
$Arg^- Hyp^- Ura^-$	10	AHU 3-12	Donor
$Arg^\circ Hyp^- Ura^-$	1	A°HU 1	Recipient & donor
$Arg^\circ Hyp^- Ura^-$	1	A°HU 2	Donor

NR = protrophic (non-requiring). Other requirements were proline (Pro^-), arginine (Arg^-), uracil (Ura^-), or hypoxanthine (Hyp^-). Arg° = arginine requirement not satisfied by ornithine.
Oc = strains producing occasional colonies on proline deficient medium and confluent growth on other auxotyping media.

Donor strains
Donor strains were selected from a collection of local isolates on the basis of auxotype.

AUXOTYPING
Auxotyping was performed as described previously.[8] Pro^-, Arg^-, Hyp^-, Ura^-, and Arg° were examined.

GENETIC TRANSFORMATION
Donor strains were cultured on chocolate agar plates (Difco blood base No 2 supplemented with 20% defibrinated horse blood at 75°C) in 5% carbon dioxide for 18 hours at 36°C. DNA was extracted by suspending the organism to about 1% (wet weight/volume) in extraction buffer (0·025% sodium dodecyl sulphate in 0·15 mol/l sodium chloride and 0·015 mol/l trisodium citrate) and incubating at 60°C for one hour. Each preparation of DNA was then dried on two chocolate agar plates.

Frozen recipient strains were thawed, plated on Kellogg's medium and incubated in 5% carbon dioxide for 18 hours at 36°C. T 1 or T 2 colonies were selectively subcultured on a second Kellogg's plate, which was incubated for 18 hours before the colony type was checked. The recipient strain was then suspended in TRIS buffered saline pH 7·2 with 2 mmol/l magnesium chloride and 2 mmol/l calcium chloride to an optical density of about 0·8 as measured by a Cecil 2292 spectrophotometer with a wavelength of 600 nm and a path length of 1 cm. This suspension was then spread on one of the chocolate plates treated with DNA and on an untreated chocolate plate (reversion control plate). As a further control each recipient strain was spread on a chocolate plate that had been treated with homologous DNA.

After being incubated for four hours at 36°C in 5% carbon dioxide, the organisms were removed from the surface of the inoculated chocolate agar plates with sterile cotton wool swabs, suspended in phosphate buffered saline, and then auxotyped. The plate treated with DNA only was incubated overnight and then checked for sterility.

Results

All preparations of DNA used in these experiments were found to be sterile. None of the selected recipient strains spontaneously reverted to prototrophy for any marker after subculture or treatment with homologous DNA. All experiments described here were performed qualitatively with no attempt to measure the incidence of transformation. Constant conditions were maintained, however, with about the same concentration of DNA and recipient organism being used in each case. The growth on a particular auxotyping plate was generally either confluent or non-existent, but in certain transformation experiments occasional (1 to 20) colonies were consistently produced. The results of the transformation experiments are given in table II.

STRAINS REQUIRING PROLINE
Two Pro^- strains (P 1 and P 2), which could easily transform one another to prototrophy, were selected from local isolates. DNA extracted from 62 other Pro^- strains (P 3-64) transformed the proline requirement of one or other of these two strains. Thus the large Pro^- group could be subdivided into organisms like P 1 (capable of transforming P 2) or like P 2 (capable of transforming P 1). In 1982 five of eight strains tested were like P 1, in 1983 nine of 18 were like P 1, and in 1985 30 of 36 were like P 1. Five β lactamase producing Pro^- strains isolated in 1985 were all like P 1.

During initial auxotyping some strains produced occasional colonies on medium free of proline but confluent growth on all other auxotyping plates. These strains, which are referred to as $Pro(oc)^-$, were not included in the experiments described above. Single colony subcultures were prepared from three $Pro(oc)^-$ strains (Poc 1-3) and the DNA was extracted and used in transformation experiments. These subcultures were also auxotyped. In all cases the transformation of P 1 produced occasional colonies on the proline deficient medium, whereas transformation of P 2 produced confluent growth. Straightforward auxotyping of these single colony subcultures consistently produced occasional colonies on medium free of proline.

DNA from two strains requiring both proline and arginine (PA 1-2) transformed P 2 but not P 1. DNA from three $Pro^-Arg^\circ Ura^-$ strains (PA°U 1-3) failed to transform either P 1 or P 2.

STRAINS WITH REQUIREMENTS FOR PROLINE, ARGININE (NOT SATISFIED BY ORNITHINE), AND URACIL
Three strains (PA°U, 1-3) of this auxogroup were used as recipients. DNA from four prototrophic strains (NR 1-4) failed to transform their requirements for arginine and uracil (table II). Furthermore, only occasional colonies were produced on the medium free of proline (table II).

STRAINS REQUIRING ARGININE
One strain from this group was selected as a recipient (A 1). It was treated with DNA from six other Arg^- strains (A 2-7). One strain (A 7) transformed it to prototrophy, but the other five did not.

TABLE II *Transformation of requirements for proline (Pro⁻), arginine (Arg⁻), hypoxanthine (Hyp⁻), and uracil (Ura⁻) to prototrophy (NR)*

Recipient strain(s)		*DNA donor strain(s)*		*Transformation to prototrophy of:*			
Designation	*Auxotype*	*Designation*	*Auxotype*	*Pro⁻*	*Arg⁻*	*Hyp⁻*	*Ura⁻*
P 1	Pro⁻	NR 1-4	NR	Yes			
P 2	Pro⁻	NR 1-4	NR	Yes			
P 1	Pro⁻	P 2	Pro⁻	Yes			
P 2	Pro⁻	P 1	Pro⁻	Yes			
P 1	Pro⁻	P 3-7	Pro⁻ (1982)	No			
P 2	Pro⁻	P 3-7	Pro⁻ (1982)	Yes			
P 1	Pro⁻	P 8-10	Pro⁻ (1982)	Yes			
P 2	Pro⁻	P 8-10	Pro⁻ (1982)	No			
P 1	Pro⁻	P 11-19	Pro⁻ (1983)	No			
P 2	Pro⁻	P 11-19	Pro⁻ (1983)	Yes			
P 1	Pro⁻	P 20-28	Pro⁻ (1983)	Yes			
P 2	Pro⁻	P 20-28	Pro⁻ (1983)	No			
P 1	Pro⁻	P 29-58	Pro⁻ (1985)	No			
P 2	Pro⁻	P 29-58	Pro⁻ (1985)	Yes			
P 1	Pro⁻	P 59-64	Pro⁻ (1985)	Yes			
P 2	Pro⁻	P 59-64	Pro⁻ (1985)	No			
P 1	Pro⁻	Poc 1-3	Pro(oc)⁻	Oc			
P 2	Pro⁻	Poc 1-3	Pro(oc)⁻	Yes			
P 1	Pro⁻	PA 1-2	Pro⁻ Arg⁻	No			
P 2	Pro⁻	PA 1-2	Pro⁻ Arg⁻	Yes			
P 1	Pro⁻	PA°U 1-3	Pro⁻ Arg° Ura	No			
P 2	Pro⁻	PA°U 1-3	Pro⁻ Arg° Ura	No			
PA°U 1-3	Pro⁻ Arg° Ura	NR 1-4	NR	Oc			
A 1	Arg⁻	NR 1-4	NR		Yes		
A 1	Arg⁻	A 2-6	Arg⁻		No		
A 1	Arg⁻	A 7	Arg⁻		Yes		
AHU 1-2	Arg⁻ Hyp⁻ Ura⁻	NR 1-4	NR		Yes	Oc	Oc
AHU 1-2	Arg⁻ Hyp⁻ Ura⁻	AHU 3-12	Arg⁻ Hyp⁻ Ura⁻		No	No	No
AHU 1-2	Arg⁻ Hyp⁻ Ura⁻	A°HU 1-2	Arg° Hyp⁻ Ura⁻		No	No	No
AHU 1-2	Arg⁻ Hyp⁻ Ura⁻	A 2-6	Arg⁻		No		
AHU 1-2	Arg⁻ Hyp⁻ Ura⁻	A 7	Arg⁻		Yes		
A°HU 1	Arg° Hyp⁻ Ura⁻	NR 1-4	NR		No*	Yes	No
A°HU 1	Arg° Hyp⁻ Ura⁻	AHU 3-5	Arg⁻ Hyp⁻ Ura⁻		No*	No	No
A°HU 1	Arg° Hyp⁻ Ura⁻	A 5-7	Arg⁻		No*		
PA°U 1-3	Pro⁻ Arg° Ura⁻	NR 1-4	NR		No		No

Oc = occasional colonies on particular auxotyping media.
*Arginine requirement not satisfied by ornithine transformed to arginine requirement satisfied by ornithine.

STRAINS REQUIRING ARGININE, HYPOXANTHINE, AND URACIL

Two strains of this auxogroup were selectively subcultured as recipient strains (AHU 1-2). Transformation of both these strains with DNA from prototrophic strains (NR 1-4) resulted in confluent growth on medium free of arginine but only occasional colonies on media free of hypoxanthine or uracil. Attempts at transforming both recipient strains with DNA from 10 other Arg⁻ Hyp⁻ Ura⁻ strains (AHU 3-12) and two Arg° Hyp⁻ Ura⁻ strains (A°HU 1-2) failed to remove the requirement for any marker. The Arg⁻ donor strain that transformed the arginine requirement of the Arg⁻ recipient strain also transformed both Arg⁻ Hyp⁻ Ura⁻ recipient strains to prototrophy for this marker. The other five Arg⁻ donor strains (A 2-6) did not.

STRAINS WITH REQUIREMENTS FOR ARGININE (NOT SATISFIED BY ORNITHINE), HYPOXANTHINE, AND URACIL

These strains only differ auxotypically from the larger Arg⁻ Hyp⁻ Ura⁻ group in that their arginine requirement cannot be satisfied by ornithine. One strain from this group was used as a recipient (A°HU 1). Its requirement for arginine (not satisfied by ornithine) was transformed to a requirement for arginine that could be satisfied by ornithine when it was treated with DNA from prototrophic (NR 1-4), Arg⁻ (A 5-7), or Arg⁻ Hyp⁻ Ura⁻ (AHU 3-6) strains, but this marker could not be transformed to prototrophy. Confluent growth was produced on medium free of hypoxanthine after treatment with DNA from prototrophic strains (NR 1-4), but no growth was evident after treatment with DNA from Arg⁻ Hyp⁻ Ura⁻ strains (AHU 3-6).

The uracil requirement could not be transformed to prototrophy.

Discussion

Local Pro⁻ strains can be divided into two genetically distinct groups by their ability to transform one of two reference strains (P 1 and P 2) to prototrophy. The defect in the proline biosynthetic pathway of local Pro⁻ Arg⁻ strains seems to be the same as the defect in P 1. When DNA from clones of individiual colonies of Pro(oc)⁻ strains was used to transform P 1, the number of prototrophic transformants was similar to the number of prototrophic colonies found in the respective clone. The same preparation of DNA however, transformed P 2 to confluent growth on the medium free of proline. This indicated that the genetic defect in Pro(oc)⁻ strains is at the same locus as the defect in P 1. Throughout this study, which took three months to conduct, P 1 never produced a revertant prototrophic colony. The reason that strains with apparently similar genetic defects should differ so noticeably with regard to reversion is not clear. Four of 14 Pro⁻ strains examined, however, have been shown to contain methyladenine in the GATC sequence,[9] and methylation of this adenine residue may increase genetic stability.[9] It will be interesting to see whether P 1 contains methylated residues in this sequence and Pro(oc)⁻ strains do not.

As in other published work,[10 11] subdivision of the large Arg⁻ Hyp⁻ Ura⁻ group by genetic transformation was not possible. The low level of transformation of the hypoxanthine and uracil markers with DNA from prototrophic strains has been reported elsewhere.[6] Juni and Heym suggest that because there are two uracil and two hypoxanthine defective loci in Arg⁻ Hyp⁻ Ura⁻ strains, two, probably unlinked, defective loci would need to be transformed to remove one of the hypoxanthine or uracil requirements.[10] This may also be the reason that transformation of the proline requirement of Pro⁻ Arg° Ura⁻ strains with DNA from prototrophic strains only produces occasioinal colonies. Furthermore, as the DNA from these Pro⁻ Arg° Ura⁻ strains failed to transform either P 1 or P 2, this auxogroup may contain both genetic defects in its proline biosynthetic pathway.

The hypoxanthine requirement of the Arg° Hyp⁻ Ura⁻ strains was transformed to confluent growth on the medium free of hypoxanthine by DNA from prototrophic strains but not from Arg⁻ Hyp⁻ Ura⁻ strains. This may mean that the hypoxanthine requirement is caused by a defect at only one locus in the Arg° Hyp⁻ Ura⁻ strains, which corresponds to one of the two hypoxanthine loci that are defective in the more common Arg⁻ Hyp⁻ Ura⁻ strains. It is not clear why the arginine and uracil requirements could not be removed by transformation in Arg° Hyp⁻ Ura⁻ strains, but it may be because these requirements are due to multiple defective loci.

Strains with a single requirement for arginine (satisfied by ornithine) could be subdivided into two groups by transformation. The Arg⁻ strain whose DNA transformed the arginine requiring recipient also removed the arginine requirement of Arg⁻ Hyp⁻ Ura⁻ strains. Thus the defect in the arginine pathway of most Arg⁻ strains is the same as in Arg⁻ Hyp⁻ Ura⁻ strains.

Genetic transformation seems to be useful in subdividing auxotypes with a single requirement for either proline or arginine. Pro⁻ strains were examined more extensively simply because they formed a larger proportion of local isolates. If Arg⁻ strains become more common it would probably be worthwhile to subdivide them by this method. The Arg⁻ Hyp⁻ Ura⁻ strains all seem to have identical defects, and transformation therefore cannot be used to subdivide them. Obviously the large prototrophic group cannot be subdivided by this method.

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References

1. Catlin BW. Nutritional profiles of *Neisseria gonorrhoeae, Neisseria meningitidis* and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. *J Infect Dis* 1973;**128**:178-94.
2. Bygdeman S, Kallings I, Danielsson D. Serogrouping and auxotyping for epidemiological study of beta-lactamase producing *Neisseria gonorrhoeae* in Sweden. *Acta Derm Venereol (Stockh)* 1981;**61**:329-34.
3. Hendry AT, Stewart IO. Auxanographic grouping and typing of *Neisseria gonorrhoeae*. *Can J Microbiol* 1979;**25**:512-21.
4. Knapp JS, Thornsberry C, Schoolnik GA, Wiesner PJ, Holmes KK, and the co-operative study group. Phenotypic and epidemiologic correlates of auxotype in *Neisseria gonorrhoeae*. *J Infect Dis* 1978;**138**:160-5.
5. Copley CG, Gough KR, Egglestone SI. Epidemiological studies on *Neisseria gonorrhoeae* isolated in the United Kingdom. *European Journal of Epidemiology* 1985;**1**:166-71.
6. Catlin BW. Genetic transformation of biosynthetically defective *Neisseria gonorrhoeae* clinical isolates. *J Bacteriol* 1974;**120**:203-9.
7. Kellogg DS, Peacock WL, Deacon WE, Brown L, Pirkle CI. *Neisseria gonorrhoeae*. 1. Virulence genetically linked to clonal variation. *J Bacteriol* 1963;**85**:1274-9.
8. Copley CG, Egglestone SI. Auxotyping of *Neisseria gonorrhoeae* isolated in the United Kingdom. *J Med Microbiol* 1983;**16**:295-302.
9. Kolodkin AB, Clark VL, Tenover FC, Young FE. High correlation of the presence of methyladenine in *Neisseria gonorrhoeae* DNA with the AHU auxotype. *Infect Immun* 1982;**36**:586-90.
10. Juni E, Heym GA. Studies of some naturally occuring auxotrophs of *Neisseria gonorrhoeae*. *J Gen Microbiol* 1980; **121**:85-92.
11. Mayer LW, Schoolnik GK, Falkow S. Genetic studies on *Neisseria gonorrhoeae* from disseminated infections. *Infect Immun* 1977;**18**:165-72.